

Supplemental Figures and Tables

Supplemental Table I. Flow cytometric staining panels.

Laser	Channel	8-Color	10-Color	11-Color	Activation
Violet 407nm	V450 ³	ViViD ¹	CD57*	CD57	HLA-DR
	V525 ³		(Alx 405) AViD ²	(Alx 405) AViD ²	(Pacific Blue) AViD ²
Blue 488nm	FITC	CD4*	TNF- α *	CD4*	Ki-67*
	PerCP-Cy5.5	CD8*	CD8*	CD8*	CD8*
Green 532nm	PE	IL-2*	IL-2*	IL-2*	Bcl-2*
	PE-Tx Rd	CD3*	CD4*	CD45RA	CD3*
	PE-Cy5	IFN- γ *	IFN- γ *	CD3*	CCR5
	PE-Cy7			CCR7	CCR7
Red 633nm	R660 ³	Perforin*	Perforin*	IFN- γ * (APC)	CD38 (APC)
	Alexa 700	(Alx647)	(Alx647)	TNF- α *	CD4*
	R780 ³	TNF- α *	Granzyme B*	CD27	CD27
			CD4* (APC-H7)	(APC-Alx750)	(APC-Alx750)

¹LIVE/DEAD Fixable Violet Dead Cell Stain (Violet Viability Dye)

²LIVE/DEAD Fixable Aqua Dead Cell Stain (Aqua Viability Dye)

³In these channels, different fluorescent dyes are used in different panels. Dyes are shown under the marker in the lists of reagents for each panel.

*Denotes intracellular reagent.

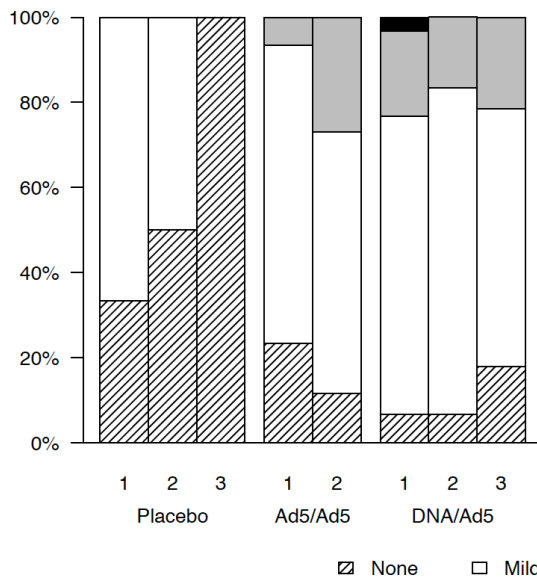
Supplemental Table II. Demographic Data.

	Ad5-Ad5	DNA-Ad5	Control
n	30	30	6*
Age (years)	28 (19-50)	28 (18-50)	35 (32-40)
Gender (%male)	16 (53%)	17 (57%)	3 (50%)
Race			
White	27 (90%)	22 (73%)	4 (67%)
Black	3 (10%)	4 (13%)	0
Hispanic	0	3 (10%)	1 (17%)
Asian	0	1 (3%)	0
Multiracial	0	0	1 (17%)
Vaccination frequency			
Day 0 (rAd5, 1 st DNA)	30 (100%)	30 (100%)	6 (100%)
Day 28 (2 nd DNA)	NA	30 (100%)	3 (50%)
Day 168 (rAd5 boost)	26 (87%)	28 (93%)	6 (100%)

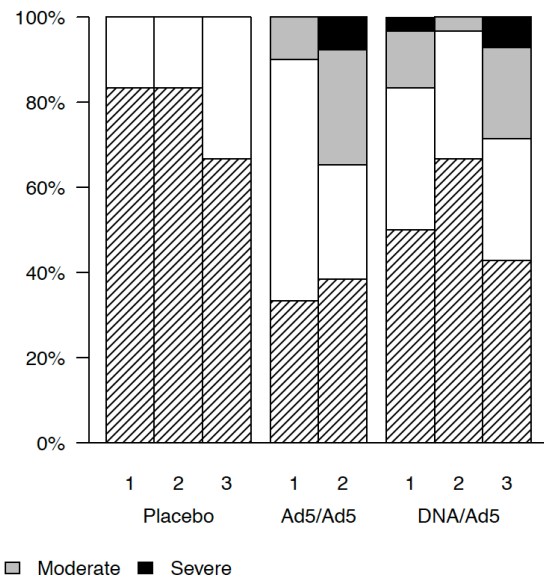
Data are number (%) or median (range)

*As a control for the Ad5-Ad5 group, three individuals received adenoviral vector final formulation buffer (FFB) at Day 0 and Day 168; as a control for the DNA-Ad5 group, three individuals received PBS at Day 0 and Day 28, and FFB at Day 168.

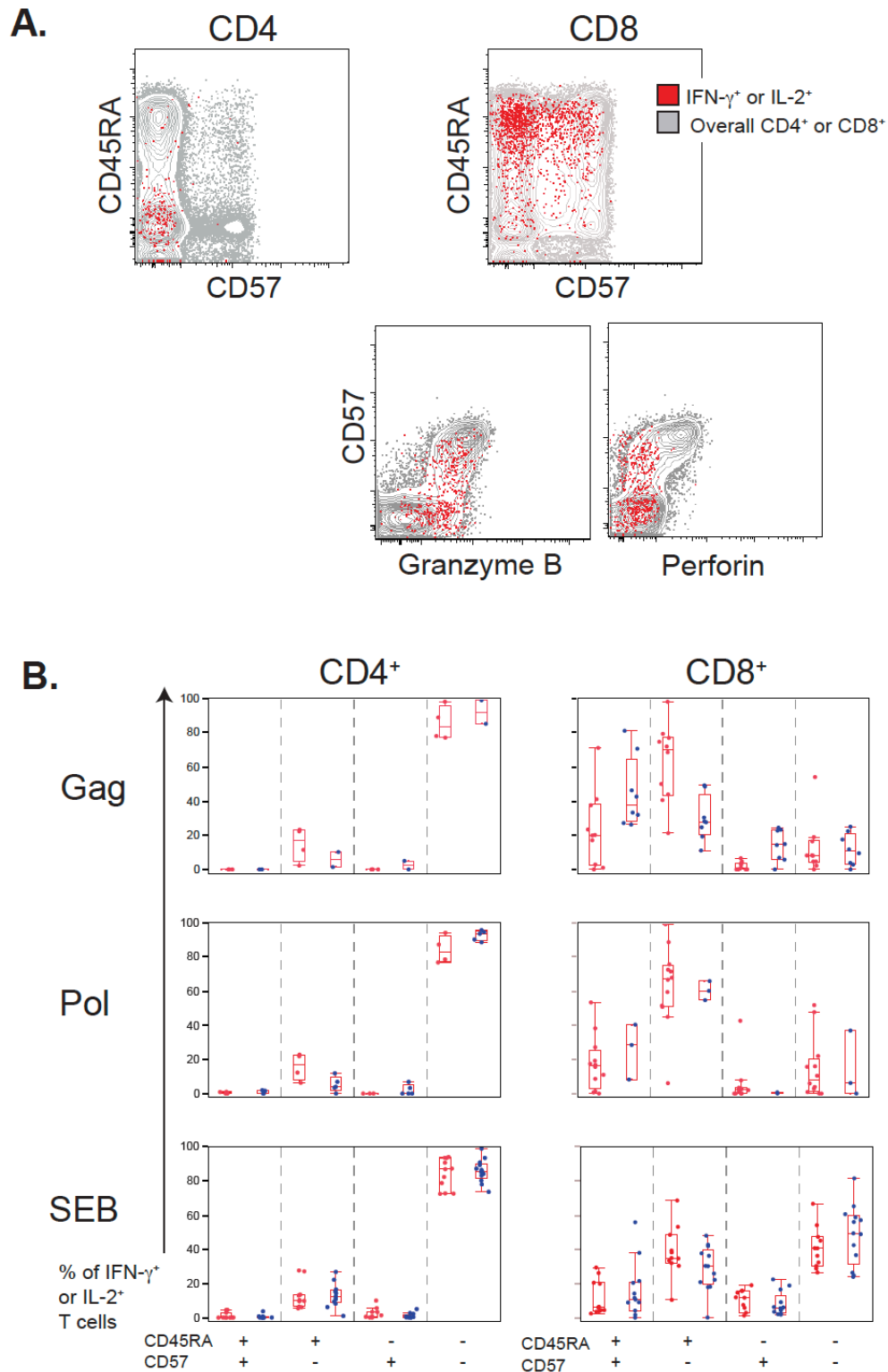
A. Maximal local reactogenicity



B. Maximal systemic reactogenicity



Supplemental Figure 1. Local and systemic reactogenicity. Shown are the percentages of participants in each study group reporting reactogenicity within 72 hours of each study vaccination. The following symptoms are considered as local reactogenicity: pain, tenderness, erythema and induration at the injection site. The following symptoms are considered as systemic reactogenicity: malaise and/or fatigue, myalgia, headache, nausea, vomiting, chills, arthralgia and above normal temperature. Within each category of local and systemic reactogenicity, the maximum level of severity for any symptom is plotted as mild, moderate or severe.



Supplemental Figure 2. Expression of CD45RA and CD57 on vaccine-induced CD4⁺ and CD8⁺ T cells. A. A representative flow cytometric staining profile for Env-specific

CD4⁺ and CD8⁺ T cells is shown for one individual from the DNA-Ad5 treatment group six months post-boost. Env-specific T cells were identified by expression of IFN- γ and/or IL-2 and are shown as red dots overlaying the overall CD4⁺ or CD8⁺ T cells (gray contours). Expression of CD45RA and CD57 are shown in the upper panels. For the CD8⁺ T cells, granzyme B, perforin and CD57 are shown in the lower panels. The Env-specific CD4⁺ T cells were negative for these markers and are not shown. B. As for Env-specific CD8⁺ T cells, Gag-specific CD8⁺ T cells show increased expression of CD57 in the DNA-Ad5 group (blue dots) compared with the Ad5-Ad5 group (red dots). The percentage of IFN- γ and/or IL-2-producing CD4⁺ or CD8⁺ T cells expressing each combination of CD45RA and CD57 in response to stimulation with Gag, Pol or SEB is shown at six months post-boost.